

As correctly indicated in the Office Action Summary, claims 1-5 and 7-23 were currently pending in this application when last examined. However, the present Supplement Amendment adds new claims 24 and 25. Applicants believe that these newly added claims further help to more precisely represent that which Applicants regard as their invention. Accordingly, claims 1-5 and 7-25 are currently pending in this application.

Support for newly added claims 24 and 25 can be found, at least, at page 15, lines 7-10. Accordingly, no prohibited new matter is believed to be introduced by this Supplement Amendment.

**Prior Art Rejections**

Claims 1, 2, 4, 5, 10-16, 18, 20, 22, and 23 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Boardman et al., *Nature*, 171:208-210 (1953) (“Boardman”).

Claims 1-5 and 7-23 also stand rejected under 35 U.S.C. §103(a) as allegedly obviousness over Boardman et al., *Nature*, 171:208-210 (1953) (“Boardman”), Sasaki et al., *J. Biochem.*, 86:1537-1548 (1979) (“Sasaki 1979”) and Sasaki et al., *J. Biochem.*, 91:1551-1561 (1982) (“Sasaki 1982”) in view of Kunin, Ion Exchange Resins, 34-39 (John Wiley & Sons, Inc., Interscience 1958) (“Kunin”), Topp et al., *J. Chem. Soc.*, Pt. 2:3299-3303 (1949) (“Topp”), Kitchener, Ion Exchangers In Organic and Biochemistry, 63-64 (Calmon and Kressman eds., Interscience Publishers, Inc. 1957) (“Kitchener”) and Guthrie, Ion Exchangers In Organic and Biochemistry, 558-559 (Calmon and Kressman eds., Interscience Publishers, Inc. 1957) (“Guthrie”).

Applicants respectfully traverse these rejections and reiterate the arguments set forth in the Reply of January 28, 2002. Applicants further traverse these rejections in light of the following remarks.

With regards to the newly added claims, Applicants reiterate the arguments set forth in the Reply of January 28, 2002 that establish that the prior art references fail to teach the binding of the protein at a pH of 5 to 9 to an uncharged resin (Reply of January 28, 2002, pages 3-5). Applicants further submit that the reference also fails to teach the selected ionizable ligands that are covalently attached to the solid support matrix which are specifically listed in the newly added claims. That is to say that Boardman is limited to a resin containing carboxyl functional groups whereas newly presented claims 56-59 recite a Markush group of functional groups which does not include a carboxyl group. It is well established that to anticipate a claim, a single prior art reference must teach, either expressly or inherently, each and every element of the claimed invention. See M.P.E.P. § 2131; Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987); Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1379, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986). Accordingly, Boardman fails to anticipate the claimed invention, because Boardman fails to teach each and every limitation of the claimed invention. Therefore, Boardman is not applicable to the newly added claims.

In further support of the argument that Boardman fails to meet the claimed limitations of all of the currently pending claims as well as the newly added claims, Applicants submit the Declaration under 37 C.F.R. § 1.132 by Simon C. Burton, one of the joint inventors of the subject matter disclosed and claimed in the present application (hereinafter the "Burton Declaration"). Applicants also submit a Declaration under 37 C.F.R. § 1.132 by Dr. Steven M. Cramer, a Professor of Chemical Engineering at Rensselaer Polytechnic Institute (hereinafter the "Cramer Declaration"). Both Drs. Burton and Cramer have worked extensively in, and are recognized experts, in the field of protein chromatography. Applicants note that executed original versions of these declarations will be promptly submitted in the following week.

The Burton Declaration discusses the prior art rejections, and in particular, the Amberlite IRC-50 carboxylate resin utilized in Boardman. This declaration also discusses experimental titration curve data generated by Burton for the carboxylate Amberlite CG50 resin. The CG50 carboxylate resin is essentially equivalent to the IRC-50 carboxylate resin except that the beads of the IRC-50 resin are much larger. It is the same resin used in the Sasaki references. The Burton Declaration compares the carboxylate resin titration data with theoretical curves calculated using the Henderson-Hasselbalch equation. See, Burton Declaration, Exhibit B.

The Burton Declaration supports the argument that the carboxylate resin of the prior art references fails to meet all of the limitations of the claimed invention. The claims of the current application contain the limitations "uncharged" and "a high or low ionic strength." Consequently, there is a requirement that the support be uncharged at high (or low) ionic strength. However, as the Burton Declaration shows, the resin in Boardman fails to meet this limitation. As shown in the titration curve in the Burton Declaration, it appears that the percentage of groups titrated at pH 5 is 20%. This curve is consistent with those found in the cited references. For instance, at high ionic strength, Boardman's data shows that at least 20% carboxyl groups are unprotonated at pH 5. This clearly contradicts the Examiner's argument at page 2 of the Office Action wherein it is stated that "[a]t a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged."

In further discussion of the Examiner's statement that "carboxylic groups are said to be wholly uncharged," the Burton Declaration makes clear that this statement may only approach reality in a distilled water titration. However, this is clearly not the case at high or low ionic strength as evident from the data in Boardman in 1M chloride. Similarly, Kunin depicts titration from pH 3.0 in 1M NaCl. Kitchener likewise reports titration from pH 4.0 in 0.1 M NaCl. The cited Sasaki articles also indicate that a pH of 4.5 or less is required to completely protonate the carboxyl groups of the resins. The product literature

as described in Rohm and Haas also reports titration between a pH of 2.5 to 4.0. In other words, it is clear that the carboxylate Amberlite IRC-50 becomes fully protonated (neutralized) only at a pH of between 2.5 and 4.0 at a high or low ionic strength. This clearly contradicts the assertions made in the Office Action and it fails to meet the limitations of the claims. Accordingly, these references fail to demonstrate binding to an uncharged resin between pH 5 and 9 at high or low ionic strength.

Furthermore, the Burton Declaration proves that the experimental titration curves disclosed in the above-cited references, in particular Kunin, Kitchener, Rohm and Haas, plus the carboxylate titration curve generated in the Burton Declaration do not fit the theoretical titration curves calculated using the simple Henderson-Hasselbalch equation. It appears that the Examiner relies on carboxylate ion exchangers and their pKas. In particular, it appears that the Examiner relies heavily on pKa followed by derivation using the Henderson-Hasselbalch equation as being superior to an experimental titration curve. However, as set forth in the Burton Declaration, pKa data is irrelevant except for theoretical calculation of the percentage of protonated carboxyl groups using the Henderson-Hasselbalch equation. This equation states that the ratio of protonated to unprotonated carboxyl groups is 1 at pH equal to the pKa; 10 at pH 1 unit below the pKa; and 0.1 at pH 1 unit above the pKa. Thus, about 90% of the titration curve should lie between the values of 1 unit either side of the pKa. However, as shown from the titration curves in the above-cited references, in particular Kunin, Kitchener, Rohm and Haas, plus the carboxylate titration curve in the Burton Declaration do not fit this. Instead, the titration range is broader, probably due to heterogeneity and neighboring group effects in a polyvalent species such as an ion exchanger. Thus, the experimental titration data discussed in the Burton Declaration makes it evident that titration of protonated carboxylates starts before pH 5. Therefore, the prior art references neither teach nor suggest the claimed invention.

The Cramer Declaration further supports the conclusion that the prior art references neither teach nor suggest binding to an uncharged resin between pH 5 and 9 at high or low ionic strength. As set forth in the Cramer Declaration, the Amberlite IRC-50 is a weakly acidic cation exchange resin which becomes fully protonated at a pH of 2.5 to 4.0 depending on the buffer salts present. Upon reviewing the titration data supplied by the manufacturer in the reference by Kunin, in the Rohm and Haas product literature, and in the data of the Burton Declaration, the Cramer Declaration concludes that the Amberlite IRC-50 carboxylate resins remain charged at pH 5 and are not fully protonated until pH less than 4.0. The Cramer Declaration even sets forth that typical weak cation exchangers used in the biotechnology industry (e.g., CM-Sephadex) have pKa's in the 3.5 to 4 range, thus requiring a low pH to become fully protonated and uncharged.

Finally, the Burton Declaration and the Cramer Declaration confirm that it is good practice to use experimental titration data rather than theoretical calculations to determine the charged state of ion exchange resins. In particular, the Cramer Declaration believes that the carboxylate titration data found in the Burton Declaration confirms that the pKa is irrelevant except for theoretical calculations of the percentage of protonated carboxyl groups using the Henderson Hasselbach equation. Moreover, the Cramer Declaration believes that these theoretical considerations should not take the place of experimental data.

Therefore, it is evident that nothing in the cited prior art references teaches or suggests the elements of the claimed invention. Absent any teaching or suggestion, the rejections maintained under 35 U.S.C. §102(b) and §103(a) are simply in error.

Moreover, as it relates to the §103(a) rejections, these references, either alone or in combination, do not provide a reasonable expectation of success to a skilled artisan that the modifications necessary to the prior art references to arrive at the claimed invention would be successful in effecting protein recovery. Thus, for the reasons noted above and for the

reasons set forth in the Reply of January 28, 2002, Applicants respectfully request the withdrawal of this rejection.

**CONCLUSION**

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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